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Evaluation of two interspecific recombinant viruses generated from two neurotropic bovine alphaherpesviruses: genomic characterization and virulence properties in the natural host

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Bovine herpesvirus 1 (BoHV-1) and bovine herpesvirus 5 (BoHV-5) are two closely related alphaherpesviruses that infect cattle. Both viruses are neurotropic but only BoHV-5 significantly replicates in the central nervous system and induces neurological disease. We previously isolated 2 interspecific recombinant viruses (R1 and R2) between BoHV-1 and BoHV-5 [1]. We report here on the precise characterization of the genetic backgrounds and on the virulence assessment of these two BoHV-1/5 recombinants in their natural host.

An exhaustive PCR/sequencing approach led us to identify the precise location of the two cross-over (CO) points where recombination events occurred between the parental strains. The first CO occurred in a 44 base pairs (bp) sequence of homology at the 3' end of the UL28 encoding genes of BoHV-1 and BoHV-5. The resulting recombinant (R1) inherited 37% of the BoHV-1 genome. The rest of its genome (63%) is homologous to BoHV-5 containing the final region of the unique long (UL) region (from UL27 to UL0.5), the internal and terminal repeats (IR and TR) and the unique short region (US). The second CO occurred in a 39 bp sequence of homology within the UL43 gene. The resulting recombinant (R2) is mainly composed of BoHV-1 sequences (86%). It is homologous to BoHV-1 in a large portion of the UL region (from UL43 to UL0.5), in the IR/TR and in the US region. BoHV-5 sequences account for 14% of its genome content.

In order to study the *in vivo* virulence of the two recombinants, six groups of 4 animals each were inoculated with the recombinant, parental and control viruses. Data obtained from the virology and serology indicated that parental BoHV-1 and R2 were fully attenuated viruses, BoHV-5 was mildly virulent in the natural host and R1 displayed an intermediate virulence pattern between its two parental strains.

In conclusion, this study provided a detailed analysis of two interspecific recombinant viruses generated from closely related alphaherpesviruses infecting the same natural host. It demonstrated that recombination can occur with very short fragments of sequence homology. Recombination is efficacious at enhancing alphaherpesvirus diversity and is susceptible to increase virus replication properties *in vivo*.

References

1. Meurens F, Keil GM, Muylkens B, Gogev S, Schynts F, Negro S, Thiry E: **Interspecific recombination between two ruminant alphaherpesviruses, bovine herpesviruses 1 and 5.** *J Virol* 2004, **78:**9828-9836.